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## Bioreactor Cultivation of a Thermophilic Bacterium Capable of Degrading BTEX

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The thermophilic bacterium, *Thermus* species ATCC 27978, which is capable of degrading the fuel-spill contaminants benzene, toluene, ethylbenzene, and the xylenes (BTEX) was cultured in 5-L bioreactors. The goal was to optimize the production of *Thermus* sp. cells possessing maximal degradative activity for their subsequent potential application in a thermally-enhanced *in situ* BTEX bioremediation process. The effects of two bioreactor cultivation modes, batch and fed batch, on the generation of BTEX-active biomass were investigated. More biomass and more thermophilic BTEX-degrading activity were produced in the fed-batch cultures than in the batch cultures. Catabolite inhibition or repression is the cause for the limited growth of *Thermus* sp. in batch bioreactors. However, the addition to the medium of *o*-cresol, a possible intermediate in BTEX metabolism, stabilized the cellular BTEX-degrading activity in such cultures. The fed-batch mode of cultivation yielded a biomass concentration of 2.5 g/L and catalytic specific activities of  $7.6 \pm 1.3$ ,  $10.1 \pm 1.9$ ,  $9.8 \pm 2.1$ ,  $2.3 \pm 0.5$ , and  $4.6 \pm 0.9$  nmol of compound degraded/mg of dry cell wt-min at 60°C for benzene, toluene, ethylbenzene, *m*-xylene, and the *o*- plus *p*-xylenes (unresolved mixture), respectively. Although the formation of BTEX-degrading activity is growth-associated, the prior rate of bioreactor growth affects the level of subsequent washed, whole-cell BTEX-degrading activity. A slow to moderate specific growth rate ( $0.02$ - $0.07$  h<sup>-1</sup>) favors the formation of cellular BTEX-degrading activity, while a high specific growth rate ( $\sim 0.16$  h<sup>-1</sup>) is detrimental to its production. Constant degrading-activity ratios among the substrates benzene, toluene, ethylbenzene, and the xylenes were observed over a wide range of incubation temperatures (45-72°C), suggesting that the biodegradation of each BTEX compound by *Thermus* sp. is catalyzed by the same intracellular enzyme system.

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